Effect of Physical Factors on Milt Quality of Malabar Grouper, Epinephelus Malabaricus in Summer and Winter Season

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ABSTRACT

A study was conducted to test the effect of physical factors such as temperature, salinity, and pH on spermatozoa motility of Malabar grouper (Epinephelus malabaricus). Mature and healthy male fishes were collected from the Red Sea during the summer and winter seasons for milt collection. Milt quality was assessed for colour, volume, sperm cell concentration, motility, and viability. Sperm motility was tested under different water temperatures, salinity, and pH media. A significant difference (P < 0.01) in sperm cell concentration (density), sperm motility (%), and viability (%) was observed between the summer and winter seasons. Changes in water temperature, pH, and salinity influenced sperm motility (%) and motility duration (min). Low pH and salinity caused adverse effects on spermatozoa motility. The results of the study are useful for the germplasm conservation of Malabar grouper, E. malabaricus.

Keywords: E. malabaricus, Milt quality, Physical factors, Spermatozoa.

1. Introduction

Physicochemical elements in the aquatic environment have an impact on the quality of sperm in marine fish (Billard & Cosson, 1988; Lahnsteiner & Mansour, 2012; Marion et al., 2014). The temperature of the water is a key factor, and for each fish species there are often optimum temperatures in which sperm motility, viability as well as fertilisation can be successful (Paul & Jayaprakash, 1996; Jezierska & Witeska, 1999; Ani et al., 2015). Deviations from this range, especially increases or decreases, can damage sperm and hamper reproduction (Aydin et al., 2012). Salinity variations can have an impact on sperm motility and fertilization rates (Revathy et al., 2016). In habitats with intensely changing salinity levels, marine fishes that are evolved to specific salinity ranges (e.g., freshwater vs. saltwater) have lower-quality sperm (Griffin et al., 1998; Tiersch & Yang, 2012). Like salinity, water pH that is too high for a specific fish species have an adverse effect on sperm motility and fertilization capacity (Alavi et al., 2004; Filiz, 2018; Sanchez et al., 2024). Acidic or alkaline environments can harm sperm cells (Sanchez et al., 2024). Even sublethal concentrations can reduce sperm motility, damage sperm morphology, and hinder fertilization success (Frommel et al., 2010). It is important to note that these are just some of the major physicochemical parameters affecting sperm quality in marine fishes.

Thorough knowledge of sperm biology and understanding of the effect of various environmental factors on the function and quality of sperm is essential for the management of breeding fish in captivity (Kowalski & Cejko, 2019). It is well known that fish sperm respond to physical and chemical stimuli in a wide variety of ways, and that what works well for one species may not work well for another (Cabrita et al., 2009). Studies on sperm biology has a significant impact on the development of methods for generating high-quality sperm and boosting the effectiveness of stock production for aquaculture species (Zhang et al., 2022). Sperm motility is a significant measure of sperm quality and is considered to be one of the most important indicators of the fertility status of males of different species (Chao et al., 1987; Linhart et al., 1995; Sambhu & Al Harbi, 2022). This is because of the functional requirement for motility of fertilizing sperm in order to reach and penetrate the egg in external fertilization (Cosson, 2019). Even though sperm quality is one of the most important factors, most fish species including some with significant economic value have little knowledge about the biology of sperm.
Malabar grouper, *Epinephelus malabaricus* is a monandric protogynous hermaphrodite species (functions first as male and later as a female), found in large numbers throughout the coastal waters of Red Sea, Saudi Arabia and are being used as a potential candidate species for aquaculture practice (Fennessy & Sadowy, 2002; Murata et al., 2014). This fish is extremely valuable and frequently referred to as the “king of fish” because of its strong demand in the market (Haq et al., 2011; 2015). Information on sperm biology is still limited, and to our knowledge, there are few studies on sperm biology (Gwo, 1993; Murata et al., 2014). The following is the first thorough research on the motility of Malabar grouper sperm. Information provided by studies into the biology of this species of fish would be useful and provide a better understanding of how best to manipulate and control the artificial reproduction of this fish for the purpose of captive breeding and commercial aquaculture production.

2. Materials and Methods

Mature male fishes of *E. malabaricus* was collected from Red Sea during summer (June-August, 2023) and winter (November, 2023-January, 2024) by fish trap and hook and line with the support of a local fisherman and brought to the wet lab of KAU Fish Farm, Faculty of Marine Science, King Abdulaziz University, Jeddah. In each season, male fish (10 nos) of uniform size were for milt collection and their individual length (35 cm) and weight (1200 g) were recorded before milt collection. Genital area of fish were wiped with clean cotton and tissue paper and gentle pressure was applied on the abdominal sides to get rid of the feacal matters and urine. Extreme care was taken to avoid contamination of milt with blood, urine or mucus. At certain cases, sufficient quantity of milt was not obtained from fish, the testis was dissected, and milt was squeezed out. A graduated pipet was used to collect milt and it was transferred immediately to a polythene bag kept in an icebox for further milt quality study.

2.1. Milt Quality

Milt quality was assessed for colour, volume, pH, sperm cell concentration, motility and viability of spermatozoa. Colour and physical appearance of milt was recorded at the time of stripping. Milt volume was calculated as the total quantum of milt drawn in a graduated pipette on a single stripping. Sperm cell concentration of the milt was assessed by using Neubar-hemocytometer method (Sukcharoen et al., 1994). Milt was diluted with saline (NaCl-0.9%) solution, and spermatozoa were counted under a compound microscope at 400× magnification and expressed as a number of spermatozoa/ml milt. Duration of motility (time period of spermatozoa show active forward movement) was studied under the activating media (sea water). One drop of milt was taken on a glass slide and mixed it with the activating medium (Dilution ratio 1:400). Slide was then observed under a pre-focused microscope (450×). Duration of motility was recorded from the time of mixing up of milt with activating media to the time up to which at least 20% of spermatozoa exhibit active forward movement. Motility percentage was calculated as Motility (%) = Number of motile spermatozoa/total number of spermatozoa × 100. Motility score was recorded based on an arbitrary scoring system developed by Agarwal (2011) and was measured as: 0%–1% (Immotile, Score-I), 1% < 25% (motility score II), 25% < 50% (motility score-III), 50% < 75% (motility score IV), and 75% < 100% (motility score V).

Sperm viability was determined by using Eosin-nigrosine dye exclusion method (Chao et al., 1987). One drop of milt was mixed with two drops of 10% nigrosine and one drop of 5% Eosin. It was then thoroughly mixed, and a thin, uniform smear was prepared on a glass slide. The slides were air-dried and observed under a microscope. Dead spermatozoa were observed as pink or red colour, and live ones in grey or ash colour. From each field, live, dead and total spermatozoa were enumerated as Viability (%) = number of live spermatozoa/total number of spermatozoa × 100.

2.2. Preparation of Physical Factors Medium

Spermatozoa motility duration (min) in different water temperatures was tested from 15 to 35°C with an interval of 5°C. Milt, media, and all the equipment used were maintained at the respective temperatures at least one hour before activation. Fresh seawater (Red Sea) with the ambient temperature was maintained as standard. Milt was mixed with the seawater (1:400 dilution) of various temperature and the duration of motility and percentage of motile spermatozoa were recorded under a compound microscope (450×). While testing the effect of temperature on sperm motility, the other physical parameters of water such as pH and salinity were maintained same as of fresh seawater. The effect of pH on the duration of motility (min) was tested in various seawater pH like 6.0, 6.5, 7.0, 7.5, 8.0, and 9.0. Milt was mixed with the respective seawater pH medium and the duration of motility and percentage of motile spermatozoa were observed under microscope. While the other parameters (temperature and salinity) were maintained as of fresh seawater. Different concentrations of saline water, such as 25, 30, 35, 40, and 45 ppt, were prepared by adding distilled water to 100% Red Sea seawater. Milt was mixed with the respective seawater pH medium and the duration of motility and percentage of motile spermatozoa were observed under microscope. A standard salinity of seawater was maintained with 100% fresh Red Sea water.

2.3. Statistical Analysis

One-way analysis of variance (ANOVA) was employed to find out the significance of the difference between the mean value of the milt quality recorded in the winter and summer seasons and the factors that affect the duration of motility and percentage of motile spermatozoa (Microsoft Excel, 2000).

3. Results and Discussion

The details of milt quality parameters recorded during the winter and summer seasons are presented in Table 1. Sperm density, sperm motility duration, percentage motility, and sperm viability were found to be significantly higher (p < 0.01) in summer when compared to winter.
season. Colour of the milt (white) was not affected by the season, but appearance changed from thick to watery in summer fishes. However, milt volume and motility score did not show the difference between the winter and summer seasons.

The effect of physical factors such as temperature, pH and salinity on sperm motility duration (min) recorded during winter and summer season are depicted in Figs. 1–3. Motility duration was found to be optimum in fresh seawater during winter and summer. it increased in summer when compared to the pared to the winter season. Results on the effect pH shows that the maximum motility duration was observed in the fresh seawater pH at 7.8. A declining trend was observed in motility when pH was increased and decreased from the optimum seawater pH level (Fig. 2). Details on the effect of salinity at different concentrations on sperm motility duration are shown in Fig. 3. Motility duration was found to lower when salinity increases and decrease from the optimum level. Enhanced motility duration was found in summer when compared to winter season. Sperm viability showed a significant difference between summer and winter, being the highest in summer than the winter season (Fig. 4).

A variety of environmental factors, such as physicochemical parameters of water, food availability, androgenic hormones, changes in daylight hours (photoperiod), disease and stressful conditions, seasonal variation, etc., can influence milt quality and sperm production in marine fishes (Fennessy & Sadovy, 2002; Marion et al., 2014; Cosson, 2019; Arianna et al., 2020). Sperm concentration, motility, and viability are the key aspects of milt quality, and a higher sperm concentration determines a higher probability of successful fertilization (Gaspare & Bryceson, 2013; Kowalski & Cezko, 2019). Result of the present study shows that the biological characteristics of the milt of E. malabaricus have significant variation in sperm cell concentration, sperm motility duration, motility percent, and viability between winter and summer seasons, and the highest performance was observed in summer. Similar enhanced sperm cell concentration and motility rates were observed in other grouper species, such as black grouper and dusky grouper (Gwo, 1993; Cabrita et al., 2009). Therefore, it is confirmed that the difference in milt quality parameters observed in Malabar grouper was due to the impact of environmental factors in the changing seasons.

Results of the present study clearly reveal that the physical factors such as water temperature, pH, and salinity influence sperm motility in Malabar grouper, E. malabaricus. According to Aydin et al. (2012), lower water temperatures generally lead to reduced sperm motility and viability, and warm water temperatures enhance sperm motility and viability in Russian sturgeon. The results of the present study show that the maximum sperm motility duration was found at a temperature 30°C than that of the lower and higher temperatures tested. Warmer temperatures increase the activity and efficiency of enzymes and other biological processes involved in sperm motility, which increases the ability of sperm to penetrate an egg during external fertilization (Billard & Cosson, 1988; Aydin et al., 2012; Lahnsteiner & Mansour, 2012). Therefore, it can be inferred that the optimum temperature for sperm motility of E. malabaricus is 30°C, and this can be a reason for the higher motility (%) recorded during the summer season than that of the winter. Jezierska and Witowska (1999) reported that high temperature increases enhanced sperm motility in carp fishes, and it varies on species and changing seasons. Based on these reports, it is confirmed that seasonal variations in water temperature can have a significant impact on Malabar grouper sperm motility.

Water pH affects the sperm motility of grouper fish, although the extent of the influence might vary depending on the species and specific pH values (Frommel et al., 2010; Haq et al., 2011; Sanchez et al., 2024). The sperm motility in grouper fish is generally higher at slightly alkaline pH levels (Kowalski & Cezko, 2019). Significant deviations from the optimal range can cause a negative impact on sperm motility (Sambhu & Al Harbi, 2022; Park et al., 2022). The results of the present study show that sperm motility duration is lowered in low pH levels and increased in higher pH at 7.8 and 8 and then decreased at 9. This indicates that the optimum pH level for sperm motility of E. malabaricus is 7.8–8. The impact of water pH on sperm motility is in many ways. Extreme pH conditions can disrupt the sperm membrane, leading to impaired motility and viability (Filiz, 2018; Park et al., 2022). The pH levels that affect sperm function during fertilization are determined by the sperm movement enzymes, which are sensitive to both high and low pH levels (He et al., 2011). Changes in pH have an unfavorable effect on the flagellum, which is responsible for sperm penetration, by reducing its ability to produce propulsive force. The sperm cells would probably suffer irreparable damage at

<table>
<thead>
<tr>
<th>#</th>
<th>Parameters</th>
<th>Winter (22°C–26°C) Mean ± SD</th>
<th>Summer (26°C–31°C) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Standard length (cm)</td>
<td>35.0 ± 2.6</td>
<td>36.2 ± 2.7</td>
</tr>
<tr>
<td>2</td>
<td>Body weight (g)</td>
<td>1216.7 ± 203.76</td>
<td>1244.9 ± 242.9</td>
</tr>
<tr>
<td>3</td>
<td>Sperm density (×10⁶ cells/ml)**</td>
<td>6.3 ± 0.2</td>
<td>6.5 ± 0.2</td>
</tr>
<tr>
<td>4</td>
<td>Milt volume (ul)**NS</td>
<td>50.1 ± 1.9</td>
<td>51.1 ± 1.9</td>
</tr>
<tr>
<td>5</td>
<td>Motility in fresh seawater (%)**</td>
<td>89.8 ± 1.4</td>
<td>92.3 ± 0.8</td>
</tr>
<tr>
<td>6</td>
<td>Motility duration (min)**</td>
<td>36.1 ± 1.4</td>
<td>38.4 ± 1.9</td>
</tr>
<tr>
<td>7</td>
<td>Sperm viability (%)**</td>
<td>82.6 ± 2.1</td>
<td>86.4 ± 4.1</td>
</tr>
<tr>
<td>8</td>
<td>Motility score</td>
<td>V</td>
<td>V</td>
</tr>
<tr>
<td>9</td>
<td>Color of milt</td>
<td>Milky white</td>
<td>Milky white</td>
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</tbody>
</table>

Note: **(p < 0.01), NS(p > 0.01), (n = 10) - One-way analysis of variance (ANOVA).

Effect of Physical Factors Grouper, Epinephelus Malabaricus in Summer and Winter Season

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Fig. 1. Effect of temperature on sperm motility duration (min) during summer and winter.

Fig. 2. Effect of pH on sperm motility duration (min) during summer and winter.

Fig. 3. Effect of Salinity on sperm motility duration (min) during summer and winter.
pH 2 and 4, which might lead to a total loss of motility and possibly even cell death (He et al., 2011). Frommel et al. (2010) reported that ocean acidification (lowering pH levels) caused decreased sperm motility and reduced sperm function in Baltic cod, Gadus morhua. The implications listed above could be the cause of the low motility rate that was seen in the current investigation at both low and high pH values. Therefore, maintaining a suitable pH level in the environment is essential for optimal sperm motility and fertilization success in E. malabaricus.

Reports show that salinity variation can significantly affect sperm motility in grouper fish (Gwo, 1993; He et al., 2011; Park et al., 2022). Sperm motility of Malabar grouper fish was high within a specific salinity range, typically between 30–35 ppt. (Gaspare & Bryceson, 2013). Significant deviations from this optimal range can negatively affect sperm motility, and the tolerances to salinity fluctuations are species-specific (Gwo, 1993; 1995; Griffin et al., 1998; Tiersch & Yang, 2012; Cosson, 2019). Grouper fish need a specific internal salt concentration to maintain the optimal cellular function (Ybanez Jr & Gonzales, 2023). Sperm motility duration of E. malabaricus in the present study showed that it increased up to 40 ppt and declined in 45 ppt. Therefore, it is suggested that 40 ppt is the optimum salinity for sperm motility in E. malabaricus. Similarly, the enhanced motility duration observed during the summer when compared to the winter season can be justified by the combined effect of water temperature, optimum pH, and salinity. Hence, maintaining a suitable salinity level in the environment is crucial for optimal sperm motility and fertilization success in grouper fish.

Sperm viability plays a crucial role in fish breeding and reproduction (Paul & Jayaprakas, 1996; Revathy et al., 2016). It is an essential quality of sperm to be alive and motile to reach and fertilize egg during external fertilization, which is the primary reproductive strategy of Malabar grouper (Haq et al., 2015). Without viable sperm, fertilization cannot occur, and that may lead to reproductive failure (Ani et al., 2015; Cosson, 2019). Sperm viability is linked to the health and quality of the resulting offspring. Studies have shown that poor sperm quality can lead to reduced fertilization success, abnormal embryo development, and lower offspring survival rates (Haq et al., 2015; Arianna et al., 2020). Viable sperm from several males can add to a fish population’s genetic variety, which is crucial for the population’s health and resistance to illnesses and environmental changes (Ani et al., 2015; Arianna et al., 2020). Sperm motility and viability are generally higher at slightly alkaline pH levels (8.0) and salinity (35 ppt) (Alavi et al., 2004; Cosson, 2019). Optimal sperm motility and viability in the present study are observed within a salinity range of 39–40 ppt. and sperm viability (%) was found to be high in summer when compared to the winter season, which is in line with the findings of Alavi et al. (2004).

4. Conclusions

The study demonstrates that the sperm quality of the Malabar grouper, E. malabaricus, is influenced by both seasonal fluctuations and physical environmental conditions. The outcomes of this study can serve as baseline information in hatcheries for induced breeding and artificial fertilization of grouper fish. More studies are required to understand the relationships between physical characteristics, nutritional state, hormone levels, and other variables affecting the sperm quality on Malabar grouper.

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Conflict of Interest

The authors declare that they do not have any conflict of interest.

References


